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=> d his

     (FILE 'HOME' ENTERED AT 15:02:49 ON 05 FEB 2003)

     FILE 'HCAPLUS' ENTERED AT 15:03:08 ON 05 FEB 2003
L1          1052 S ((LEVY S?) OR (LEVY,S?) OR (LEVY, S?))/AU,IN
L2           891 S ((WONG R?) OR (WONG,R?) OR (WONG, R?))/AU,IN
                 E MCMUR/AU,IN
                 E MCMURRY/AU,IN
L3            42 S E32-E35
L4             2 S (L1 OR L2 OR L3) AND (BLR OR BETA(W)LACTAM(W)358)

=> d stat que
L1          1052 SEA FILE=HCAPLUS ((LEVY S?) OR (LEVY,S?) OR (LEVY, S?))/AU,IN
L2           891 SEA FILE=HCAPLUS ((WONG R?) OR (WONG,R?) OR (WONG, R?))/AU,IN
L3            42 SEA FILE=HCAPLUS ("MCMURRY L M"/AU OR "MCMURRY LAURA"/AU OR
                   "MCMURRY LAURA M"/AU OR "MCMURRY LAURA M"/IN)
L4             2 SEA FILE=HCAPLUS (L1 OR L2 OR L3) AND (BLR OR BETA(W)LACTAM(W)3
                   58)


=> d ibib abs hitrn l4 1-2

L4   ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         2001:763064  HCAPLUS
DOCUMENT NUMBER:          135:329199
TITLE:                    The BLR gene of Escherichia coli affecting
                          sensitivity to antibiotics acting on peptidoglycan
                          synthesis
INVENTOR(S):              Levy, Stuart B.; McMurry, Laura M.
PATENT ASSIGNEE(S):       Trustees of Tufts College, USA
SOURCE:                   PCT Int. Appl., 104 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.   DATE
     ---------------   ----  --------       ---------------   --------
     WO 2001077176      A2   20011018       WO 2001-US11363   20010406
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
             HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
             LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,
             RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN,
             YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
             DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
             BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
     US 2002051982      A1   20020502       US 2001-828456    20010406
PRIORITY APPLN. INFO.:                     US 2000-195505P   P  20000406
                                           US 2000-218380P   P  20000714
AB   A novel 358 base pair sequence encoding a membrane protein that affects
     susceptibility to antibiotics that affect peptidoglycan synthesis in
     microbes is described. BLR nucleic acid and polypeptide mols.
     are provided. In addn., screening assays to identify agents that modulate
     BLR activity are described. The role of the gene in the
     resistance to .beta.-lactam antibiotics is demonstrated by mutational
```

anal. Suppressible mutants demonstrate that the gene product plays a role in the resistance.

L4 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:569768 HCAPLUS
DOCUMENT NUMBER: 134:37716
TITLE: "Intergenic" **blr** gene in Escherichia coli encodes a 41-residue membrane protein affecting intrinsic susceptibility to certain inhibitors of peptidoglycan synthesis
AUTHOR(S): **Wong, Rebecca S. Y.; McMurry, Laura M.; Levy, Stuart B.**
CORPORATE SOURCE: Center for Adaptation Genetics and Drug Resistance and the Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA, 02111, USA
SOURCE: Molecular Microbiology (2000), 37(2), 364-370
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In the annotation of genomic sequences, small open reading frames (ORFs) are often neglected, particularly if they have no homol. to other ORFs or proteins. A mini-TnphoA insertion in a 602 bp "intergenic" region of the Escherichia coli chromosome at genomic nucleotide 1702674 gave rise to a membrane-bound PhoA fusion protein and a two- to fourfold increase in the intrinsic susceptibility to a wide spectrum of beta-lactam antibiotics without affecting beta-lactamase activity or susceptibility to tetracycline, chloramphenicol, gentamicin or quinolones. Susceptibility was also increased to cycloserine and bacitracin, but not to fosfomycin or valinomycin; these drugs, like beta-lactams, inhibit peptidoglycan synthesis, although by different mechanisms. A clone bearing only 358 bp of this "**blr**" region restored resistance to the parental level. Two amber mutations in the clone prevented such restoration and were counteracted by an amber suppressor, proving that the active species is a protein. The **Blr** protein has 41 amino acids, with a single predicted transmembrane helix, but no clear homol. to any other protein. A transcriptional start exists 39 bp upstream from the translational start. The membrane location of **Blr** suggests that it may be part of an efflux pump or involved in murein metab. The results indicate that genes for other very small functional proteins may lie within "intergenic" regions.
REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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show files
File 155:MEDLINE(R) 1966-2003/Feb W1
         (c) format only 2003 The Dialog Corp.
File   5:Biosis Previews(R) 1969-2003/Feb W1
         (c) 2003 BIOSIS
File  34:SciSearch(R) Cited Ref Sci 1990-2003/Jan W4
         (c) 2003 Inst for Sci Info
File  35:Dissertation Abs Online 1861-2003/Jan
         (c) 2003 ProQuest Info&Learning
File  71:ELSEVIER BIOBASE 1994-2003/Feb W1
         (c) 2003  Elsevier Science B.V.
File  73:EMBASE 1974-2003/Jan W4
         (c) 2003 Elsevier Science B.V.
File  94:JICST-EPlus 1985-2003/Nov W3
         (c)2003 Japan Science and Tech Corp(JST)
File 144:Pascal 1973-2003/Jan W4
         (c) 2003 INIST/CNRS
File 340:CLAIMS(R)/US Patent 1950-03/Feb 04
         (c) 2003 IFI/CLAIMS(R)
File 345:Inpadoc/Fam.& Legal Stat 1968-2002/UD=200304
         (c) 2003 EPO
File 351:Derwent WPI 1963-2003/UD,UM &UP=200308
         (c) 2003  Thomson Derwent
File 357:Derwent Biotech Res. _1982-2003/Feb W1
         (c) 2003 Thomson Derwent & ISI
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
         (c) 1998 Inst for Sci Info
File 440:Current Contents Search(R) 1990-2003/Feb 06
         (c) 2003 Inst for Sci Info
?ds

Set     Items   Description
S1         17   (BLR OR BETA(W)LACTAM358 OR BETA(W)LACTAM(W)358) AND ANTIB-
               IOTIC?(S)(RESISTANCE? OR MODULAT? OR ACTIV?)
S2          7   RD (unique items)
?t2/3 ab/1-7
>>>No matching display code(s) found in file(s): 345

 2/AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

10848337   20392465   PMID: 10931331
   Intergenic' blr gene in Escherichia coli encodes a 41-residue membrane
protein affecting intrinsic susceptibility to certain inhibitors of
peptidoglycan synthesis.
  Wong R S; McMurry L M; Levy S B
  Center for Adaptation Genetics and Drug Resistance and the Department of
Molecular Biology and Microbiology, Tufts University School of Medicine,
136 Harrison Avenue, Boston, MA 02111, USA.
  Molecular microbiology (ENGLAND) Jul 2000, 37 (2) p364-70, ISSN
0950-382X Journal Code: 8712028
  Contract/Grant No.: GM51661; GM; NIGMS
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  In the annotation of genomic sequences, small open reading frames (ORFs)
are often neglected, particularly if they have no homology to other ORFs or
proteins. A mini-TnphoA insertion in a 602 bp 'intergenic' region of the
Escherichia coli chromosome at genomic nucleotide 1702674 gave rise to a
membrane-bound PhoA fusion protein and a two- to fourfold increase in the
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intrinsic susceptibility to a wide spectrum of beta-lactam antibiotics without affecting beta-lactamase activity or susceptibility to tetracycline, chloramphenicol, gentamicin or quinolones. Susceptibility was also increased to cycloserine and bacitracin, but not to fosfomycin or valinomycin; these drugs, like beta-lactams, inhibit peptidoglycan synthesis, although by different mechanisms. A clone bearing only 358 bp of this ' blr ' region restored resistance to the parental level. Two amber mutations in the clone prevented such restoration and were counteracted by an amber suppressor, proving that the active species is a protein. The Blr protein has 41 amino acids, with a single predicted transmembrane helix, but no clear homology to any other protein. A transcriptional start exists 39 bp upstream from the translational start. The membrane location of Blr suggests that it may be part of an efflux pump or involved in murein metabolism. The results indicate that genes for other very small functional proteins may lie within 'intergenic' regions.

2/AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

09597774 98005680 PMID: 9345766
Blepharismin produced by a protozoan Blepharisma functions as an antibiotic effective against methicillin-resistant Staphylococcus aureus.
Pant B; Kato Y; Kumagai T; Matsuoka T; Sugiyama M
Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Japan.
FEMS microbiology letters (NETHERLANDS) Oct 1 1997, 155 (1) p67-71, ISSN 0378-1097 Journal Code: 7705721
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A ciliated protozoan, Blepharisma japonicum, produces a photosensitive red pigment, blepharismin (BLR). This study showed that the pigment inhibits the growth of Gram-positive bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA) resistant to arbekacin (ABK), which is the most effective aminoglycoside antibiotic against MRSA and used world wide. Although the minimum inhibitory concentration (MIC) of BLR to the ABK-resistant MRSA strain was 6.25 micrograms/ml in dark, it was decreased to 1.25 micrograms/ml by irradiation with white light of 65 W/m2 for 30 min, suggesting that the antibacterial activity of BLR is photoactivated. Our findings suggested that the antibacterial activity of BLR in dark is due to inhibition of protein synthesis. In addition, we found that BLR is bactericidal and enhances synergistically the antibacterial activity of ABK.

2/AB/3 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

13561833 BIOSIS NO.: 200200190654
Accumulation of a polyisoprene-linked amino sugar in polymyxin-resistant Salmonella typhimurium and Escherichia coli. Structural characterization and transfer to lipid A in the periplasm.
AUTHOR: Trent M Stephen; Ribeiro Anthony A; Doerrler William T; Lin Shanhua ; Cotter Robert J; Raetz Christian R H(a)
AUTHOR ADDRESS: (a)Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710**USA E-Mail: raetz@biochem.duke.edu
JOURNAL: Journal of Biological Chemistry 276 (46):p43132-43144 November 16, 2001

MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Polymyxin-resistant mutants of Escherichia coli and Salmonella typhimurium accumulate a novel minor lipid that can donate 4-amino-4-deoxy-L-arabinose units (L-Ara4N) to lipid A. We now report the purification of this lipid from a pss- pmrAC mutant of E. coli and assign its structure as undecaprenyl phosphate-alpha-L-Ara4N. Approximately 0.2 mg of homogeneous material was isolated from an 8-liter culture by solvent extraction, followed by chromatography on DEAE-cellulose, C18 reverse phase resin, and silicic acid. Matrix-assisted laser desorption ionization/time of flight mass spectrometry in the negative mode yielded a single species (M-H)- at m/z 977.5, consistent with undecaprenyl phosphate-alpha-L-Ara4N (Mr=978.41). 31P NMR spectroscopy showed a single phosphorus atom at -0.44 ppm characteristic of a phosphodiester linkage. Selective inverse decoupling difference spectroscopy demonstrated that the undecaprenyl phosphate group is attached to the anomeric carbon of the L-Ara4N unit. One- and two-dimensional 1H NMR studies confirmed the presence of a polyisoprene chain and a sugar moiety with chemical shifts and coupling constants expected for an equatorially substituted arabinopyranoside. Heteronuclear multiple-quantum coherence spectroscopy analysis demonstrated that a nitrogen atom is attached to C-4 of the sugar residue. The purified donor supports in vitro conversion of lipid IVA to lipid IIA, which is substituted with a single L-Ara4N moiety. The identification of undecaprenyl phosphate-alpha-L-Ara4N implies that L-Ara4N transfer to lipid A occurs in the periplasm of polymyxin-resistant strains, and establishes a new enzymatic pathway by which Gram-negative bacteria acquire antibiotic resistance .

2001

2/AB/4 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent
(c) 2003 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 10108386 IFI Acc No: 2002-0051982 IFI Acc No: 2002-0014216
Document Type: C
NOVEL BLR MOLECULES AFFECTING ANTIBIOTIC SUSCEPTIBILITY
Inventors: Levy Stuart B (US); McMurry Laura M (US); Wong Rebecca S Y (CA)
Assignee: Unassigned Or Assigned To Individual
Assignee Code: 68000
Publication (No,Date), Applic (No,Date):
US 20020051982 20020502 US 2001828456 20010406
Publication Kind: A1
Priority Applic(No,Date): US 2001828456 20010406
Provisional Applic(No,Date): US 60-195505 20000406; US 60-218380 20000714

Abstract: A novel 358 base pair sequence encoding a membrane protein that affects susceptibility to antibiotics that affect peptidoglycan synthesis in microbes is described. BLR nucleic acid and polypeptide molecules are provided. In addition, screening assays to identify agents that modulate BLR activity are described.

2/AB/5 (Item 1 from file: 351)
DIALOG(R)File 351:Derwent WPI
(c) 2003 Thomson Derwent. All rts. reserv.

014190201
WPI Acc No: 2002-010898/200201
XRAM Acc No: C02-002722
New membrane protein, designated Beta Lactam - 358 polypeptides, that affect susceptibility to antibiotics which affect peptidoglycan synthesis in microbes, useful for identifying modulators for treating infections
Patent Assignee: TUFTS COLLEGE (TUFT); LEVY S B (LEVY-I); MCMURRY L M (MCMU-I); WONG R S Y (WONG-I)
Inventor: LEVY S B; MCMURRY L M; WONG R S Y
Number of Countries: 095 Number of Patents: 003
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 200177176	A2	20011018	WO 2001US11363	A	20010406	200201	B
AU 200189318	A	20011023	AU 200189318	A	20010406	200213	
US 20020051982	A1	20020502	US 2000195505	P	20000406	200234	
			US 2000218380	P	20000714		
			US 2001828456	A	20010406		

Priority Applications (No Type Date): US 2000218380 P 20000714; US 2000195505 P 20000406; US 2001828456 A 20010406
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 200177176	A2	E	104	C07K-014/705	
AU 200189318	A			C07K-014/705	Based on patent WO 200177176
US 20020051982	A1			C12Q-001/68	Provisional application US 2000195505
					Provisional application US 2000218380

Designated States (National): AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW

Abstract (Basic): WO 200177176 A2
Abstract (Basic):
NOVELTY - An isolated beta lactam - 358 polypeptide (membrane protein) (I) comprising an amino acid sequence 50% identical to a sequence (S1) of 41 amino acids fully defined in the specification, a fragment of 15 contiguous amino acids of S1, a naturally occurring homolog of S1, or amino acids encoded by a sequence 50% identical to a sequence (S2) of 481 nucleotides fully defined in the specification, is new.
DETAILED DESCRIPTION - (I) comprises an amino acid sequence 50% identical to S1, a fragment of 15 contiguous amino acids of S1, a naturally occurring homolog of S1, where the homolog is isolated from a pathogenic bacterium and is encoded by a nucleic acid molecule which hybridizes to S2, or amino acids encoded by a sequence 50% identical to S2.
INDEPENDENT CLAIMS are also included for the following:
(1) an isolated polynucleotide (II) comprising a nucleotide sequence which is at least 50% identical to S2, or their complement, which is from:
(a) a nucleic acid molecule (NAM) comprising a fragment of at least 100 nucleotides of S2 or its complement;
(b) a NAM encoding a polypeptide (I); and
(c) a NAM encoding a fragment of a polypeptide comprising S2, where the fragment comprises at least 15 contiguous amino acids residues of S2;
(2) an isolated polynucleotide which hybridizes to (II) under

stringent conditions;
(3) an isolated polynucleotide which is complementary to (II);
(4) an isolated polynucleotide comprising (II) and a nucleotide sequence encoding a heterologous polypeptide;
(5) a vector (III) comprising (II);
(6) a host cell (IV) transfected with (III);
(7) preparation of (I);
(8) an antibody (Ab) which selectively binds to (I); and
(9) an agonist or antagonist of (I).
ACTIVITY - Antibacterial.
MECHANISM OF ACTION - (I) affects susceptibility to antibiotics that inhibit peptidoglycan synthesis in microbes; vaccine. No supporting data is given.
USE - (I) is useful for identifying compounds that modulate antibiotic resistance in a microbe. The method comprises contacting (I) preferably present in a microbial cell e.g. Escherichia coli, with a test compound in vitro, determining the ability of the test compound to modulate the activity or expression of (I), preferably by measuring the effect of the test compound on the ability of the microbial cell to grow in the presence of an antibiotic that affects peptidoglycan synthesis selected from beta lactam, cycloserine and bacitracin, or measuring the efflux of the test compound or a marker compound from the cell, and selecting those compounds that modulate the activity of (I), to identify compounds that modulate antibiotic resistance , where (I) is heterologous to the cell. The method can optionally be performed utilizing (II), which comprises contacting (II) with a test compound, determining the ability of the test compound to bind to (II) and selecting those compounds that bind to (II) to identify compounds that modulate antibiotic resistance . (II) is useful for identifying a protein that interacts with (II). The method comprises contacting (II) with a microbial extract under conditions which allow interaction of components of the extract with (II), and measuring the ability of (II) to interact with the components for identifying a protein that binds to (II) (claimed). (II) is useful for identifying a compound that modulates the ability of (II) to interact with BLR binding polypeptide, by contacting (II) and a beta lactam resistance (BLR) binding polypeptide with a test compound under conditions and measuring the ability of the compound to modulate the interaction of (II) with the BLR binding polypeptide to identify a compound that modulates the ability of a BLR nucleotide sequence to interact with a BLR binding polypeptide. (I) is useful identifying a compound that modulates the ability of a BLR polypeptide to interact with a BLR binding polypeptide comprising contacting (I) and a BLR binding polypeptide with a test compound under conditions which allow interaction of the compound with (I) and the BLR binding polypeptide, and measuring the ability of the compound to modulate the interaction of (I) with the BLR binding polypeptide to identify a compound that modulates the ability of (I) to interact with a BLR binding polypeptide (all claimed). The BLR modulating agents which include (I), (II), polypeptide homologs, agonists or antagonists of (I) and antibodies is useful in treatment of infection particularly infection with organism resistant to antibiotics that affect peptidoglycan synthesis, screening assays, use in vaccines and diagnostic assays. (II) is useful for expressing (I) e.g. in a host cell in gene therapy applications, to detect BLR mRNA in a biological sample or a genetic alteration in a BLR gene and to modulate BLR activity . Ab is useful for detecting and isolating (I), for regulating the bioavailability of (I) and for modulating (I) activity .
pp; 104 DwgNo 0/1

2/AB/6 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.

0279110 DBR Accession No.: 2002-03251 PATENT
New membrane protein, designate beta - lactam - 358 polypeptides, that affect susceptibility to antibiotics which affect peptidoglycan synthesis in microbes, useful for identifying modulators for treating infections - plasmid pRW23C and plasmid pRW23D-mediated gene transfer, expression in host cell, antibody, agonist and antagonist for antibiotic - resistance organism infection diagnosis, vaccine and gene therapy
AUTHOR: Levy S B; McMurry L M
CORPORATE SOURCE: Medford, MA, USA.
PATENT ASSIGNEE: Tufts-Coll. 2001
PATENT NUMBER: WO 200177176 PATENT DATE: 20011018 WPI ACCESSION NO.: 2001-010898 (200101)
PRIORITY APPLIC. NO.: US 218380 APPLIC. DATE: 20000714
NATIONAL APPLIC. NO.: WO 2001US11363 APPLIC. DATE: 20010406
LANGUAGE: English
ABSTRACT: A beta - lactam - 358 membrane protein (I) with a 41 amino acid protein sequence fully defined encoded by a 358 bp DNA sequence (II) fully defined is claimed. Also claimed are: a vector containing (II); a host cell transfected with the vector; production of (I) by culturing the host cell and recovering (I) form the culture medium; an antibody that selectively binds to (I); and an antagonist or agonist of (I). In an example, the (I)- resistance gene was isolated and amplified using polymerase chain reaction. Plasmid pRW23C and plasmid pRW23D were constructed and a spectinomycin- resistance cassettes was inserted. (I) can be used identifying compounds that modulate antibiotic - resistance in a microbe e.g. Escherichia coli. (II) can be used for identifying a compound that modulates the ability of (II) to interact with (I)-binding protein. The above can be used for infection in organism that are resistant to antibiotics that affect peptidoglycan synthesis, for drug screening, diagnosis, vaccine and gene therapy. (104pp)

2/AB/7 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2003 Thomson Derwent & ISI. All rts. reserv.

0185590 DBR Accession No.: 95-12411 PATENT
New pre-coelenterazine peptide derived from jellyfish green fluorescent protein - fluorescence reporter gene expression in Escherichia coli using vector plasmid pTU132
AUTHOR: Ward W; Chalfie M
PATENT ASSIGNEE: Ward W; Chalfie M 1995
PATENT NUMBER: WO 9521191 PATENT DATE: 950810 WPI ACCESSION NO.: 95-283729 (9537)
PRIORITY APPLIC. NO.: US 192158 APPLIC. DATE: 940204
NATIONAL APPLIC. NO.: WO 95US1425 APPLIC. DATE: 950203
LANGUAGE: English
ABSTRACT: A pre-coelenterazine peptide (I), which is a modified form of Aequorea victora green fluorescent protein (GFP) with Tyr at position 65, is claimed. Also new are: a polynucleotide (II) encoding (I); expression vector containing (II); organisms transformed with the vector; recombinant coelenterazine (III) produced by expressing (II); and purified luciferyl sulfate (LS) derived from (III). The protein sequence (238 amino acids) of 65Tyr GFP and the DNA sequence (994 bp) encoding it are specified. Preferably, (I) contains residues 1-228 of mutated GFP, especially all 238 amino acids, and has N-terminal Met-Ala, 80-Gln, 172 Glu and (a) 100-Phe, 108-Thr, 141-Leu and 219-Val,

or (b) 100-Tyr, 108-Ser, 141-Met and 219-Ile. Vectors containing (II) may also contain a promoter plus an antibiotic - resistance selectable marker gene. The vector is preferably plasmid pTU132 (ATCC 75666). Transformed cells are animal, bacterial, plant or insect cells, particularly Escherichia coli BLR (DE3) or SMC2 (ATCC 69553), or a squid giant neuron. Expression of (II) is used for production of (III) as a bioluminescence indicator. (55pp)

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L4 ANSWER 1 OF 6 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:418609 BIOSIS
DN PREV200000418609
TI 'Intergenic' blr gene in Escherichia coli encodes a 41-residue membrane protein affecting intrinsic susceptibility to certain inhibitors of peptidoglycan synthesis.
AU Wong, Rebecca S. Y.; McMurry, Laura M.; Levy, Stuart B. (1)
CS (1) Center for Adaptation Genetics and Drug Resistance and the Department of Molecular Biology and Microbiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA, 02111 USA
SO Molecular Microbiology, (July, 2000) Vol. 37, No. 2, pp. 364-370. print. ISSN: 0950-382X.
DT Article
LA English
SL English
AB In the annotation of genomic sequences, small open reading frames (ORFs) are often neglected, particularly if they have no homology to other ORFs or proteins. A mini-TnphoA insertion in a 602 bp 'intergenic' region of the Escherichia coli chromosome at genomic nucleotide 1702674 gave rise to a membrane-bound PhoA fusion protein and a two- to fourfold increase in the intrinsic susceptibility to a wide spectrum of **beta-lactam** antibiotics without affecting beta-lactamase activity or susceptibility to tetracycline, chloramphenicol, gentamicin or quinolones. Susceptibility was also increased to cycloserine and bacitracin, but not to fosfomycin or valinomycin; these drugs, like **beta-lactams**, inhibit peptidoglycan synthesis, although by different mechanisms. A clone bearing only **358** bp of this 'blr' region restored resistance to the parental level. Two amber mutations in the clone prevented such restoration and were counteracted by an amber suppressor, proving that the active species is a protein. The Blr protein has 41 amino acids, with a single predicted transmembrane helix, but no clear homology to any other protein. A transcriptional start exists 39 bp upstream from the translational start. The membrane location of Blr suggests that it may be part of an efflux pump or involved in murein metabolism. The results indicate that genes for other very small functional proteins may lie within 'intergenic' regions.
AB. . . a membrane-bound PhoA fusion protein and a two- to fourfold increase in the intrinsic susceptibility to a wide spectrum of **beta-lactam** antibiotics without affecting beta-lactamase activity or susceptibility to tetracycline, chloramphenicol, gentamicin or quinolones. Susceptibility was also increased to cycloserine and bacitracin, but not to fosfomycin or valinomycin; these drugs, like **beta-lactams**, inhibit peptidoglycan synthesis, although by different mechanisms. A clone bearing only **358** bp of this 'blr' region restored resistance to the parental level. Two amber mutations in the clone prevented such restoration. . .
IT . . .
Parts, Structures, & Systems of Organisms
chromosome
IT Chemicals & Biochemicals
Blr; bacitracin: antibacterial - drug, enzyme inhibitor - drug; **beta-lactam** antibiotics: antibacterial, enzyme inhibitor - drug; beta-lactamase; chloramphenicol: antibacterial - drug, enzyme inhibitor - drug; cycloserine: antibacterial - drug, enzyme. . .